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Rats Clearing a Vaginal Infection by *Candida albicans* Acquire Specific, Antibody-Mediated Resistance to Vaginal Reinfection

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Oophorectomized, estrogen-treated rats were susceptible to experimental vaginal infection by *Candida albicans*. After spontaneous clearing of the primary infection, the animals were highly resistant to a second vaginal challenge with the fungus. The vaginal fluid of *Candida*-resistant rats contained antibodies directed against mannan constituents and secretory aspartyl proteinase(s) of *C. albicans* and was capable of transferring a degree of anti-*Candida* protection to naive, nonimmunized rats. This passive protection was mediated by the immunoglobulin fraction of the vaginal fluid and was substantially abolished by preabsorption of the vaginal fluid with *C. albicans*, but not with *Saccharomyces cerevisiae*, cells. Vaginal anti-mannan antibodies were also produced by active immunization with heat-killed cells of *C. albicans* or with a mannan extract when administered via the vaginal route. The protection conferred was comparable to that resulting from clearing of the primary infection. In summary, the data suggest that acquired anticandidal protection in this vaginitis model is mediated at least in part by antibodies, among which those directed against the mannan antigen(s) might play a dominant role.

Although vaginal candidiasis is a widespread, common disease affecting about one-third of all women at least once in their life span (20, 21), the mechanisms of pathogenicity and anticandidal immune response at the vaginal level remain substantially obscure. It is sufficiently clear that an occasional, acute attack of the disease does not require a special predisposition by the host. However, a minority of women, estimated to be about 5% of all those of childbearing age, suffer from recurrent attacks of the disease. Some authors have suggested that these women have local, if not systemic, specific immune deficiency leading to a high susceptibility to the disease (25, 26).

To understand the fungal and host factors involved in the pathogenesis of candidal vaginitis, estrogen-dependent mouse or rat models of vaginal infections have been employed (7, 19, 23). In particular, using the mouse model, Fidel and collaborators have presented a considerable set of experimental data linking vaginal infection with elicitation of specific, systemic cell-mediated immunity (CMI) to *Candida albicans*, although this intense CMI response could not be correlated with anti-*C. albicans* protection mechanisms acting at the vaginal level (12–14).

Using oophorectomized, estrogen-treated rats, we obtained circumstantial evidence about the role of aspartyl-proteinase(s), a putative virulence factor of *C. albicans* in the pathogenesis of the vaginal infection (4, 7). We have also recently demonstrated that the vaginal environment has unique, though still undefined, properties allowing for antigenic variations and hypha formation even by nongerminative mutants of *C. albicans* (6, 9). Finally, we have shown that anti-idiotypic antibodies are elicited to a substantial level in the vaginas of rats immunized intravaginally or subcutaneously with an anti-yeast killer toxin monoclonal antibody. These antibodies mimicked the anti-*C. albicans* killer toxin in protecting against vaginal candidiasis (18).

In this study, we have used the rat model in an attempt to understand whether the spontaneous clearing of the initial infection that is typical in rodent vaginal candidiasis affects a subsequent rechallenge with the fungus. We focused particularly upon antibody responses at the vaginal level and investigated the effect of this response on anticandidal protection.

MATERIALS AND METHODS

Microorganisms and growth conditions. The yeasts used throughout this study were the vaginopathic *C. albicans* SA-40 and *Candida parapsilosis* SA-23, both isolated from the vaginal secretion of women with acute vaginitis (4, 8), as well as a chemically mutagenized strain of *C. albicans* (13B10) which is avirulent and nongerminative (kindly provided by F. Bistoni, University of Perugia, Perugia, Italy). A strain of *Saccharomyces cerevisiae* isolated from the vagina of an asymptomatic subject was also used.

All yeasts were maintained in sterile distilled water and subcultured on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.). Unless otherwise specified, experiments were performed with strain SA-40. For the experimental infection, each strain from SDA subcultures was grown to the stationary phase in YEPD medium (yeast extract, 10 g/liter; neopeptone, 20 g/liter; dextrose, 20 g/liter) at 28°C, and harvested by centrifugation (3,500 × *g*), washed, and suspended to the required number in phosphate-buffered saline (PBS; Oxoid; Unipath Ltd., Basingstoke, Hampshire, England).

Animals. Oophorectomized female Wistar rats, 80 to 100 g (body weight; Charles River Breeding Laboratories, Calco, Italy), were used throughout this study. Estrogen treatment and both initial infection and rechallenge were performed exactly the same as described previously (7).

The animal experimentation referred to in this paper was approved by the ad hoc committee of the Istituto Superiore di Sanità, Rome, Italy.

Experimental rat vaginitis. All rats were maintained under pseudoestrus by injection of estradiol benzoate (Benzatron; Samil, Rome, Italy; 0.5 mg subcutaneously) every 2 days. Six days after the first estradiol dose, the animals were inoculated intravaginally with 10^7 yeast cells in 0.1 ml of saline solution, administered to each animal with a syringe equipped with a multipurpose calibrated tip (Combitip; PBI, Milan, Italy). Vaginal fluid was taken from each animal every 2 days with a calibrated (1-μl) plastic loop (Dispoinoc; PBI). The contents of each loop were vigorously suspended in 0.1 ml of PBS and aliquots were streaked over SDA plates containing 0.05% (wt/vol) chloramphenicol and incubated at 28°C for 48 to 72 h. One vaginal sample per rat per culture was evaluated, and the rat

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was considered infected when at least 1 CFU was present in one loop of vaginal fluid, i.e., a count of $\geq 10^3$ CFU/ml (6, 7). At intervals during the vaginal infection, the colonies developed on SDA were reidentified by standard diagnostic criteria (4, 7). As reported elsewhere (6, 7), this procedure offered a reproducible and consistent determination of fungal burden in the vagina. Some fluids were also stained by the periodic acid-Schiff–van Gieson method for microscopic examination.

Collection of vaginal fluid washes. For serological analysis and transfer experiments (see below), samples of vaginal fluids were taken at regular intervals from each animal either before or after the intravaginal challenge with yeast cells. The rat vaginal cavity was washed by gentle injection-aspiration of 0.5 ml of PBS, repeated three times with the same 0.5 ml and always performed by the same operator. The final fluid sample collected was centrifuged at 3,500 × *g* for 15 min in a refrigerated Biofuge, and the supernatant was assayed for vaginal antibodies as described below.

Antibodies against *C. albicans* constituents. The presence of antibodies directed against mannan antigens or the aspartyl-proteinase(s), the latter of which is a putative virulence factor of *C. albicans* (4, 5, 21), was assayed in the vaginal wash by a previously described enzyme-linked immunosorbent assay (ELISA) (7, 8, 24). A low-protein (<1.5% by weight) mannan extract (MP) (24) was used as a coating antigen for the detection of anti-mannan antibodies. It was shown previously that this extract was representative of the predominant, cell surface-associated *C. albicans* polysaccharide and glycoprotein antigens (3). Briefly, 200 μl of the MP solution (5 μg/ml in 0.2 M sodium carbonate) was dispensed into the wells of a polystyrene microtitration plate which was kept overnight at 4°C. After three washes with Tween 20-PBS buffer, triplicate wells were given 1:2 dilutions of vaginal fluids, and the plates were incubated for 1 h at room temperature. Each well was washed again five times with Tween 20-PBS buffer, and predetermined optimal dilutions of alkaline phosphatase-conjugated, sheep anti-rat immunoglobulin G, (IgG) IgM, or IgA (obtained from Serotec Ltd., Kidlington, Oxford, United Kingdom) were added. For the anti-proteinase antibody assay in vaginal fluid, the same ELISA was used, except that a highly purified, non-mannan-containing preparation of acid proteinase enzyme was used as the coating antigen (4, 7). The enzyme preparation was kindly provided by P. A. Sullivan (Dunedin, New Zealand). Bound alkaline phosphatase was detected by the addition of a solution of *para*-nitrophenylphosphate in diethanolamine and magnesium chloride buffer and by reading the A_{405} with an automated microreader (Titertek Multiscan; Skatron, Oslo, Norway) blanked against air.

Preliminary experiments of ELISA reactivity between mannan and proteinase with affinity-purified anti-proteinase (7) or anti-mannan (9) antibodies, respectively, demonstrated the absence of any cross-reaction. A vaginal fluid was considered positive for a determined antibody when the ELISA reading was greater than 2 standard deviations from the mean value of the well coated with the same antigen and including the vaginal fluid of *C. albicans* 13B10-infected rats which did not contain antibodies (usual optical density O.D. readings, in the range of 0.14 to 0.22).

Passive transfer of vaginal fluids. Vaginal washes were collected on day 45 following the primary vaginal infection, and 0.5 ml of these fluids (pooled from at least three rats) was injected intravaginally into naive, unimmunized, oophorectomized, and estradiol-treated rats. After 30 min, each recipient rat was inoculated with *C. albicans* at the usual challenging dose of 10^7 cells, and *C. albicans* vaginal CFU were monitored as described above. Control rats received either the vaginal wash from animals clearing a primary infection with *C. parapsilosis* (8) or the fluid from rats infected with the nonvaginopathic strain 13B10 of *C. albicans*. In some experiments, the vaginal wash was separated into immunoglobulin (Ig) and non-Ig fractions by fractional precipitation with ammonium sulfate by routine biochemical procedures (16). The separated fractions were brought to identical protein concentrations before transfer to unimmunized animals.

Active immunization. Two groups of six rats each, under the usual estrogen treatment, were given intravaginally 10^7 heat-inactivated (100°C, 2 h) cells of *C. albicans* or *S. cerevisiae* or mannan extract (MP; 200 μg) emulsified in 0.1 ml of complete Freund adjuvant (at time zero and at 7 days). Subsequently, the same number of yeast cells or the same dose of mannan, suspended in PBS, was administered on days 14 and 21. Another group of six rats received adjuvant and PBS only. After each administration, the rats were held supine for 10 min to facilitate vaginal absorption of the injected material. On day 30, all animals were challenged intravaginally with 10^7 CFU of *C. albicans*, and vaginal *C. albicans* counts were enumerated as described above. The experiment was repeated, and vaginal fluids were harvested for assessment of anti-*C. albicans* antibodies.

Statistical analysis. The significance of CFU differences was assessed by Student's *t* test.

RESULTS

Outcome of primary and secondary rat vaginal infections. In preliminary experiments, we reassessed the susceptibility of oophorectomized, estradiol-treated rats to a vaginal infection by *C. albicans* under the conditions selected for the present study. Following an intravaginal challenge with 10^7 yeast cells, $>10^5$ candidal CFU/ml of vaginal fluid could be found in the first week postinfection; the vaginal burden then slowly declined to $\leq 10^3$ fungal cells at 28 to 34 days. Vaginal smears prepared at intervals during infection demonstrated sustained hyphal growth starting 1 day postinfection and extending through day 7 to 14. However, no fungal cells were detected by day 28. Thus, the results of these experiments confirmed previous data (4, 6, 7, 9).

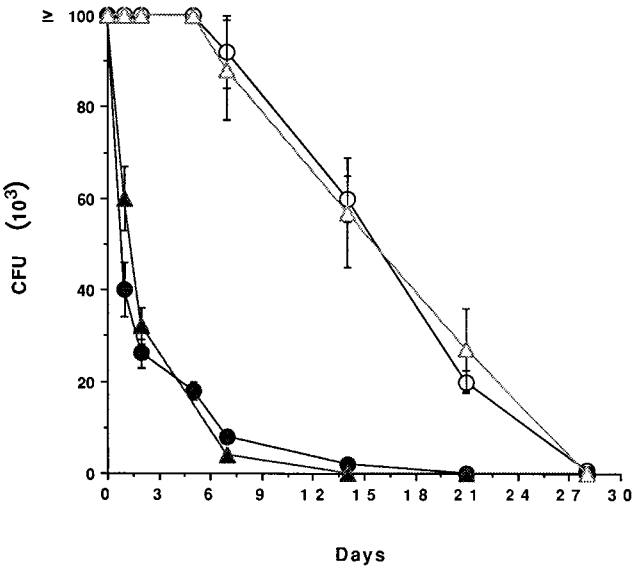

FIG. 1. Outcome of vaginal infection in oophorectomized, pseudoestrus rats. Rats were challenged on day 0 with *C. albicans* SA-40 (○) or 13B10 (▲); in each group, six rats were used. After 45 days, the rats challenged primarily with the vaginopathic strain SA-40 were rechallenged with the same strain (●), whereas those challenged primarily with strain 13B10 were rechallenged with SA-40 (△). Vaginal CFU were enumerated at the indicated times. Bars indicate the standard errors of the means. Starting 1 day postchallenge, and excluding the last day of measurement (day 28), there was always a highly significant difference ($P < 0.01$, Student's *t* test) between the candidal CFU of the rats infected primarily with *C. albicans* SA-40 and those of animals challenged primarily with *C. albicans* 13B10 or SA-40-infected rats rechallenged after healing the initial infection. The experiment was repeated twice with an identical number of rats for each group, and similar results were obtained.

Figure 1 shows the outcome of primary and secondary challenges after clearing the first infection. The animals infected with the vaginopathic strain SA-40 cleared the second infection much more rapidly than the first, as the end point, $<10^3$ CFU/ml of vaginal fluids, was approached at the end of the first week and no vaginal CFU could be detected at the end of the second week (Fig. 1). Thus, these rats were essentially as resistant to rechallenge as those inoculated primarily with an avirulent, germ-tube-deficient mutant of *C. albicans* (Fig. 1) (see also reference 19) or a nonvaginopathic yeast isolate (*S. cerevisiae*) (6). In contrast, rapid clearing of the nonvaginopathic strain 13B10 did not confer resistance to a second challenge with SA-40. When the animals resistant to vaginal reinfection were challenged intravenously with *C. albicans*, they were as susceptible to lethal systemic disease as normal, naive animals (data not shown).

Protection against *C. albicans* rechallenge by transfer of vaginal fluids from rats clearing the primary infection. In an attempt to understand the local factors and mechanisms conferring protection against vaginal reinfection by *C. albicans*, we examined the potential of the cell-free vaginal fluid harvested from rats clearing the first infection to transfer protection to uninfected, naive rats. The vaginal fluids from rats challenged

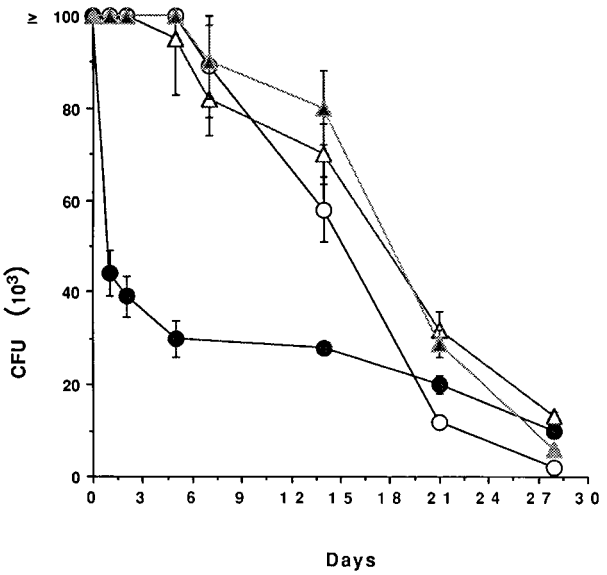

FIG. 2. Outcome of vaginal infection in oophorectomized, estradiol-treated rats challenged primarily with *C. albicans* SA-40 (○) or injected intravaginally with the vaginal fluids of rats clearing a primary infection with SA-40 (●), rats challenged with strain 13B10 of *C. albicans* (▲), or rats clearing a primary infection with *C. parapsilosis* (△), before challenge with *C. albicans* SA-40. In each group, six rats were used. Starting day 1 postinfection, and until the day 13, there was always a highly significant difference ($P < 0.01$, Student's t test) between the vaginal CFU of rats injected with vaginal fluids from rats clearing the initial infection with strain SA-40 and the CFU of all other rats. The data are from a representative experiment of three performed with similar results (see also the text).

TABLE 1. Anti-mannan and anti-proteinase antibodies in the vaginal fluids of rats during primary infection with *C. albicans*

Day	Isotype	No. positive/total[a]	
		Anti-mannan	Anti-proteinase
0	IgM	0/9	0/6
	IgG	0/9	0/6
	IgA	0/6	0/6
14	IgM	3/9	0/9
	IgG	3/9	0/9
	IgA	2/6	2/6
28[b]	IgM	8/9	2/6
	IgG	9/9	4/6
	IgA	5/6	4/5

[a] The antibody response was evaluated as the number of positive vaginal fluid samples of the total number examined, as assayed by ELISA. For the definition of positive fluid, see Materials and Methods. The data are from one experiment of two performed with similar results.

[b] The means ± standard deviations of actual optical density readings of the positive vaginal fluids on day 28 were as follows: 0.43 ± 0.16, 0.57 ± 0.21, and 0.54 ± 0.18 for anti-mannan IgM, IgG, and IgA, respectively, and 0.48 ± 0.12, 0.64 ± 0.13, and 0.53 ± 0.09 for anti-proteinase IgM, IgG, and IgA, respectively.

with a nonvaginopathic, nongerminative variant (13B10) of *C. albicans* (see also Fig. 1) as well as fluids from rats infected with *C. parapsilosis* (8) served as controls. In a typical experiment of three performed with similar results (Fig. 2), the administration of vaginal fluid from rats clearing the initial infection with *C. albicans* caused an immediate reduction of the vaginal implant of the same strain in naive, previously uninfected rats. This resistance was not observed in rats given the control vaginal fluids (Fig. 2). However, the duration of resistance was shorter than that conferred upon the rats by the clearance of the first candidal infection (Fig. 1) or that conferred by active immunization with inactivated cells of *C. albicans* or mannan extract (see below).

Antibodies in the vaginal fluids of rats infected with *C. albicans*. The data described above prompted us to examine whether antibodies against *C. albicans* were present in the vagina of protected rats. To this aim, the vaginal fluids of infected rats were harvested at 0, 14, and 28 days postinfection and examined by an ELISA with a mannan extract (24) or a highly purified aspartyl proteinase enzyme preparation (4, 7) from *C. albicans*. The vaginal fluids of uninfected or *C. albicans* 13B10-infected rats served as controls (in one of the two experiments). Table 1 shows the results of one of the two independent determinations of anti-mannan or anti-proteinase antibodies, expressed as the number of positive vaginal fluid samples from each single rat examined. Anti-mannan antibodies were detected in a proportion of vaginal fluids on day 14 postinfection. The number of ELISA-positive fluids increased thereafter to reach 100% anti-mannan-positive fluids (IgG) on day 28, with simultaneous presence of all isotype classes examined. Anti-proteinase antibodies (mostly IgG and IgA) were also consistently detected in the majority of infected rats on day 28. The ELISA results were confirmed by Western blotting (immunoblotting), which showed the presence of antibody reactive with a 44-kDa proteinase band as well as with a high-molecular-mass, polydisperse mannan (data not shown). When the vaginal fluids from uninfected rats or from rats infected with the avirulent mutant *C. albicans* 13B10 were examined, no anti-mannan or anti-proteinase antibodies of any isotype were detected.

Protection against infection conferred by the Ig fraction of the vaginal fluids of primarily infected rats. To assess any cause-effect relationship between the vaginal anticandidal antibodies and protection conferred by passive transfer of immune vaginal fluids, we performed experiments whereby the vaginal fluid from infected rats (day 35 postinfection), which contained antibodies against *C. albicans* constituents, was separated into Ig and non-Ig fractions by fractional precipitation with ammonium sulfate. After desalting, the two fractions were brought to the same total protein concentrations and injected into the vaginal cavity of naive rats 1 h before challenge with *C. albicans*. An aliquot of the Ig fraction was adsorbed with heat-inactivated *C. albicans* or *S. cerevisiae* cells before vaginal injection. Table 2 shows the CFU per milliliter of vaginal fluid recovered 1, 2, or 7 days after infection by *C. albicans*. While the control, nonpretreated rats had the usual fungal burden in the vagina, the animals preinjected with the Ig fraction of the immune vaginal fluid had a sharp decrease in vaginal CFU starting 1 day postinfection. This rapid decay of fungal burden did not occur in the rats pretreated with the non-Ig fraction of the vaginal fluid, the numbers of vaginal CFU of which were very much similar to those of the control rats. Finally, when the vaginal Ig fraction was preadsorbed with heat-inactivated *C. albicans* but not *S. cerevisiae* cells, the protection conferred by this fraction was greatly reduced, if not abolished, in keeping with removal of anti-mannan antibodies from the fraction (Table 2). This suggests that anti-*C. albicans* Ig contributes to protection of the vaginal tissue against infection with *C. albicans*.

Protective effects of active immunization with mannan extract from *C. albicans*. Since the data described above suggested that anti-mannan antibodies could be one active constituent of the protection against vaginal reinfection, we devised a series of experiments involving active immunization with the same mannan extract from *C. albicans* used as antigen to detect anti-*Candida* antibodies in infected rats. The results

TABLE 2. Effect of passive transfer of Ig and non-Ig fractions of immune vaginal fluids on vaginal CFU in week 1 following *C. albicans* infection[a]

Expt group	*Candida* vaginal CFU (10^3)[b]		
	1 day	2 days	7 days
Control rats	>100	>100	>100
Rats receiving Ig fraction of immune vaginal fluid	52 ± 7	46 ± 11	36 ± 10
Rats receiving non-Ig fraction of immune vaginal fluid	>100	>100	88 ± 18
Rats receiving *C. albicans*-preadsorbed[c] Ig fraction of immune vaginal fluid	>100	82 ± 12	79 ± 7
Rats receiving *S. cerevisiae*-preadsorbed Ig fraction of immune vaginal fluid	56 ± 11	52 ± 4	44 ± 5

[a] Each experimental group consisted of four oophorectomized, estradiol-treated rats which received an amount of material equalized for protein concentration (as determined by a commercial Bio-Rad assay). There was, at any time point, a statistically significant difference ($P < 0.01$, Student's *t* test) between the vaginal CFU of control rats (or rats receiving the non-Ig fraction of the vaginal fluid) and those of the animals receiving the Ig fraction. There was also a statistically significant difference ($P < 0.01$) at each time point examined between the vaginal CFU of control rats and those of the animals receiving *S. cerevisiae*-preadsorbed vaginal fluid (but not those receiving *C. albicans*-preadsorbed fluid), as assessed by Student's *t* test. The experiment was repeated twice, with similar results.

[b] Expressed as mean ± standard deviation.

[c] Preadsorption with *C. albicans* or *S. cerevisiae* cells was carried out with 0.5 ml of the Ig fraction with 10^8 heat-inactivated (100°C, 2 h) fungal cells, and the adsorption was repeated twice. No ELISA-detectable anti-mannan antibodies were present in the Ig fraction after adsorption on *Candida* cells (optical density reading, < 0.20 from >1.0 before adsorption).

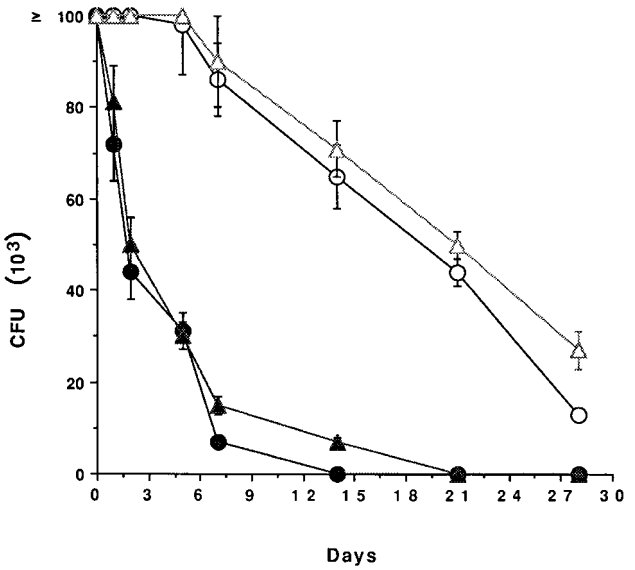

FIG. 3. Outcome of vaginal infection in rats immunized intravaginally with heat-killed *C. albicans* SA-40 cells (●) or a mannan extract (MP) of *C. albicans* (▲), given adjuvant only (△), or immunized with *S. cerevisiae* (○), before infection with *C. albicans* SA-40. Six rats per group were used. Starting day 1 postinfection, there was always a statistically significant difference ($P < 0.01$, Student's *t* test) between the vaginal CFU of the animals immunized with whole cells of *C. albicans* or mannan and those of the animals immunized with cells of *S. cerevisiae* or nonimmunized (adjuvant only). No statistically significant difference was detected between *Candida* cell- or mannan-immunized rats at any time point. The results are from a representative experiment of two performed with similar results.

were compared with those obtained by immunization with whole inactivated cells of *C. albicans* or *S. cerevisiae*. In keeping with the model adopted, active immunization was performed by the intravaginal route. Figure 3 shows the results obtained in a typical experiment of two performed with similar results. Immunization with either the mannan extract or heat-killed *C. albicans* cells resulted in an accelerated clearance of *C. albicans* from the vaginal cavity, whereas rats immunized with *S. cerevisiae* or administered adjuvant only had prolonged periods of clearance. The kinetics of *C. albicans* clearance from the vaginas of the rats actively immunized with either whole fungal cells or mannan extract resembled the curve of fungal clearance from the vagina of the animals protected following recovery from the initial infection rather than that of vaginal immune fluid- or Ig fraction-transferred animals (compare data of Fig. 1, 2, 3, and Table 2). Intravaginal immunization with mannan extract or heat-killed cells of *C. albicans* resulted in the elicitation of specific anti-mannan antibodies in both cases (data not shown).

DISCUSSION

The rat vaginal model has generally been used in studies assessing antimycotic activity in vivo or potential virulence factors and intravaginal growth characteristics of *C. albicans* (4, 6, 19, 20, 21, 23). In a seemingly similar mouse model (not requiring oophorectomy but only estrogen administration), Fidel and collaborators have recently demonstrated the elicitation of systemic *C. albicans*-specific CMI, particularly of the Th-1 type, in intravaginally infected animals (12–14). There was no correlation, however, between the level of adaptive anticandidal CMI expressed by lymphocytes of the peripheral blood or lymph nodes and the extent of vaginal infection, suggesting that systemic CMI was probably uncoupled from vaginal immunity (12, 14). The possible role of antibodies in the infection was not addressed. Some of us have shown recently that antibodies mimicking an anticandidal killer toxin from *Pichia anomala* could be obtained by vaginal immunization with the appropriate idiotype vaccine, i.e., an anti-killer toxin monoclonal antibody. These anti-idiotypic antibodies were present in the serum and in the vaginal cavity (in this case, they were mostly IgA) and were highly protective against a subsequent vaginal challenge with *C. albicans* (18).

In this paper, we show that after clearance of the primary vaginal infection by *C. albicans*, oophorectomized, estrogen-treated rats acquire a resistance to vaginal reinfection with the fungus, as demonstrated by a much-accelerated clearance of the new infectious inoculum. This resistance could be transferred passively to *C. albicans*-nonimmunized rats by the vaginal fluid of immune rats. Resistance to reinfection after clearing of the primary infection has also been observed in the mouse vaginitis model (11).

Together, these findings document for the first time that the clearance of candidal vaginitis confers some immunity to local reinfection. We show here that the acquired resistance to reinfection may be mediated ultimately by soluble, rather than cellular, factors elicited or recruited in the vaginas of infected rats.

Although we have not precisely identified these factors, the following needs to be considered for a plausible interpretation of our results. First, only the vaginal fluids of immunized rats contain antibodies, particularly of both IgG and IgA isotypes, directed against two important antigens and putative virulence factors of *C. albicans*, namely, cell surface mannan and the aspartyl proteinase. Second, the protective factor(s) belongs to the Ig fraction of the vaginal fluid and can be removed specif-

ically by adsorption with whole *C. albicans* cells. Third, active immunization via the vaginal route with a mannan extract from *C. albicans* provides significant protection from the infectious challenge. This is associated with elicitation of anti-mannan antibodies in the vagina and substantially equals the protection achieved by immunization with whole *C. albicans* cells, which are known to express antigenic mannan on their surface (3), as well as the protection achieved after clearance of the primary infection. All of these facts constitute a consistent body of circumstantial evidence that anticandidal antibodies, especially anti-mannan antibodies, are likely those playing a role in the protection. The lack of protection in the rats which were administered anti-*C. parapsilosis* antibody-rich vaginal fluids also demonstrates that the putative protective factor recognizes a specific structure of *C. albicans.*

The protective effects of passive transfer of immune vaginal fluids (or the Ig fraction thereof) were almost immediate (they were usually highest on day 1 postchallenge) but, in contrast to active immunization, showed a tendency to vanish with time. This is an expected difference between active and passive direct antibody-mediated protection. Of interest also is the fact that the rats with acquired resistance to vaginal challenge with *C. albicans* after primary infection are not protected from systemic challenge, in keeping with the observation that mannan extracts do not confer protection to animals challenged systemically with *C. albicans* (15). This strengthens the concept that anticandidal immunity in superficial infections may differ substantially from systemic immunity to the fungus and may require stimulation of local immunity. Along this line, a special consideration should be devoted to the high immunogenicity coupled to efficient protection possessed by *C. albicans* antigens and other immunogens when injected intravaginally, as shown here and elsewhere (18).

Since no inflammatory cells are usually seen in the vaginal smears of infected rats (6, 23), it is unlikely that the antibodies against *C. albicans* exert protection through opsonization of the fungal cell. More likely is a direct role, e.g., an anti-adhesion or anti-germ-tube formation. In this context, the studies showing the importance of mannan moieties of mannoprotein adhesins (and the inhibitory effects of anti-adhesin antibodies) on *C. albicans* attachment to host cells (1, 2, 5, 17) are relevant. This phenomenon is considered important in the pathogenesis of vaginitis (20–22).

Clearly, the vaginal fluids of the rats clearing the initial infection could contain other antibodies and possibly unknown factors of natural or adaptive immunity which may help in acquiring a protective state. Particularly, anti-proteinase IgA antibodies were found in the vaginal fluids of the majority of the rats described above. We have previously suggested a role for proteinase in the pathogenesis of vaginitis (4, 7), and the presence of vaginal anti-proteinase antibodies was expected from the previously described active production of this enzyme during infection (7). Unfortunately, it has been impossible to date to separate antibodies in sufficient quantity to perform transfer experiments with anti-proteinase- or anti-mannan-specific antibodies only. Since the cells of *C. albicans* used in the absorption experiments were not induced to express proteinase (7), the reduced ability of the *C. albicans*-preadsorbed, immune vaginal fluid to transfer protection favors the interpretation that a main protective role in our model could be played by the anti-mannan antibodies, without detracting from the possibility that anti-proteinase antibodies could also be involved.

The results of the experiments of active immunization with mannan, although in line with a role for anti-mannan antibodies, must be interpreted cautiously since mannan has been shown to activate nonspecific natural immunity and cytokine production (3, 10). It seems unlikely, however, that cytokines produced by natural immunoeffectors or T cells are present in the vaginal fluid 35 days postchallenge and are specifically adsorbed by *C. albicans* cells. Moreover, there was no evidence of recruitment of inflammatory cells into the vaginal cavity during infection. The exact nature of the protective, soluble factors in rat vaginal infection by *C. albicans* is being investigated further, using monoclonal antibodies directed against immunodominant *Candida* antigens and by searching for the presence of cytokines produced by immunocompetent cells or keratinocytes during the infection.

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